Medullary spots and their cause

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(WITH PLATES 10 AND 11)

Introduction

It is practically an established fact now that the common types of medullary spots or "pith flecks" so frequently found in the wood of trees and shrubs, are due to the mining of insect larvae in the cambium. The reliability of such marks as aids in determining the wood of forest trees therefore depends upon the relative distribution of the different species of cambium miners and the plants they infest.

The injurious effect of the mining on the health of the host usually depends upon the relation of the mined or eaten area of cambium to the area of the portion that is uninjured. In the case of large trees the effect on the host is usually slight while on a small tree or shrub a very heavy infestation may result in marked stunting of the plant's growth. Some woods may become worthless for finishing purposes owing to the presence of numerous occluded mines. Perhaps the chief pathological interest in this matter lies in following the response of the affected regions of the bark which results in proliferations to occlude the channels left by the foraging miners, and also in the development of new cambium over the outside of the mines.

This article is an addition to and a continuation of a short paper* written a few years ago, in that more of the details of the host's response as well as the complete life history of the miner discussed in the latter part of the earlier paper (pp. 63–65) are given. It is based on notes and specimens accumulated during the years 1910–12.

Some additional and more recent literature

All of the important earlier literature on the botanical side of the subject of medullary spots was reviewed in some detail in the

* Medullary spots: a contribution to the life history of some cambium miners. N. Y. Agr. Exp. Sta. Tech. Bull. 15: 49–65. 1910.

above cited paper before giving the life history of the *Ribes* miner (*Opostega nonstrigella*). A little later Record* published a very similar review of the same literature and added some observations on the occurrence of medullary spots in other hosts. Another review of the early literature was given in a later paper by Brown† in which he also records additional hosts and gives some details regarding the mines and miners. Five very excellent plates give interesting information about the mines. However, in his discussion of the occlusion of the channels made by the insects, occurs an error that should not be left unmentioned: "The larva destroys only those cells in its immediate path through the inner bark. . . . As the cambium layer moves outward radially, the passage left by the larva increases in diameter. For this reason, when growth is very rapid, the pith-fleck spots are larger than when it is slow." The error in these statements may be readily shown by quoting from a few lines below out of the same paper: "The healing process proceeds mainly from the bast pith-ray cells in the bark." This shows that Brown's observations were correct and that the channels once made are not moved outward radially by further growth of cells on the wood-side of the wound. In other words, it is unlikely that subsequent growth could widen a channel made by a cambium miner and it is very apparent that the mines are made narrower by the enlargement of the normal cells on both sides. Plate 4 of his paper shows how the channels are filled mainly by proliferations from the rays on the bark side.

The first to determine the most important stages in the life history of a cambium miner was Nielsen.‡ He studied the larva in all its stages, the pupa as well as the imago, though the egg was not found. The miner proved to be the larva of a fly, *Agromyza carbonaria*. He found that the larvae fed in the cambium until autumn and then entered the ground, where they passed the winter as pupae. The adult flies emerge in May.

* Record, S. J. Pith flecks or medullary spots in wood. For. Quar. **9**: 244–251. 1911.

† Brown, H. P. Pith-ray flecks in wood. Forest Ser. U. S. Dept. Agr. Cir. 215: 5–15. *pl. 1–5.* 1913.

‡ Nielsen, J. C. Über die Entwicklung von *Agromyza carbonaria* Zett., der Urheber der "Markflecken." Zool. Anzeiger **29**: 221–222. 1905; Zoologische Studien über die Markflecke. Zool. Jahrb. **23**: 725–737. 1906.

At first the larva has but one hook and well-developed girdles of plates which increase in number per segment from the first to the last: the first segment having one, and the last or twelfth, from seven to nine. This is also characteristic of the *Prunus* miner as given in my former paper. The second hook eventually appears on the left of the first. It is very small and lies in the curve of the large one as is characteristic of an *Agromyza* larva. The length of the larva increases from about three millimeters to twenty millimeters before emerging from the bark to pupate in the ground. A girdle of small spines is developed on the second segment. They project caudad. Before the end of larval life the number of plate girdles is much reduced; the skin becomes thicker and wrinkles crosswise. The pupa is three to four millimeters long, barrel-shaped, and has the spiracles at the ends on the dorsal side.

Some of Nielsen's figures of the causal insect were later reproduced by Tubeuf* in an article on medullary spots. He made the interesting observation, also frequently noted by the writer, that the miners are present only in such trees of a species which have active cambium throughout most of the growing season. In the autumn they were found most numerous in trees growing in wet places. He secured only the larval stages of the miners.

Greene† recently gave an account of the pupation of a single larva of the river birch miner discussed by Brown and described the adult that emerged from it. The fly was thought to be *Agromyza pruinosa*, and the opinion is expressed that possibly it is the only species in America producing medullary spots. The latter, of course, is a premature and very unlikely assumption even as regards the dipterous insects that mine in the cambium of woody plants; besides it has long since been shown to be incorrect as regards cambium miners in general. At least one other cambium miner is a lepidopterous insect (*Opostega nonstrigella*). Owing to the fact that our knowledge of the subject is so very fragmentary and incomplete it is evident that we are not in position to generalize as yet. In fact if Greene's statement on page 474 of the above

* Tubeuf, K. von. Über die Zellgänge der Birke und anderer Laubhölzer. Naturwiss. Zeit. Forst- und Landwirt. **6**: 235–241. 1908.

† Greene, C. T. The cambium miner in river birch. Jour. Agr. Research **1**: 471–474. 1914.

paper, to the effect that "*Agromyza pruinosa* remains in the pupal stage in the ground during winter" applies to the fly which he and Brown studied it certainly differs from the one discussed in this paper. The *Prunus* miner, as noted above, was found to hibernate in the cambium of the host during the winters of 1910–11 and 1911–12 in the larval stage and to pupate early the following summers.

LIFE HISTORY OF THE PRUNUS MINER

On pages 63–65 of my paper on medullary spots cited above are described the early stages of a cambium miner that feeds in *Prunus* and *Crataegus* but does not attack *Salix*. The observations upon which that discussion was based extended from August 15 to November 15, 1910; or to the time when the cambium had dormancy forced upon it by the early cold weather of western New York. The larvae continued mining until nearly mid-November but were mostly still under six millimeters in length when they ceased feeding.

In 1911 the first careful examination and the first large collection of miners was made on May 21. They had apparently been active for some time. The mines were most numerous in the thicker parts of the infested shrubs. The channels were larger than the ones made by the same larvae in the 1910 growth, as was readily seen by tracing them back to the previous year's growth. The larvae still fed with their lateral sides toward the wood and bark, but the end turns in their mines were both clockwise and counter-clockwise. In the stem near the ground the return trips were often only a decimeter or two in length resulting in a zigzag course, but some of them still were making trips 1.6 meters in length. About a fourth of the larvae had left their hosts by boring their way out through the bark. The holes were still open and could be readily seen once they had been found. The larvae usually came out through the bark a centimeter or more after making a lower turn. Some exit holes were found as high as a meter above but most of them were within two to four decimeters of the ground; others entered the cambium of the crown and roots before mining their way out. In many instances mines had been made downward to about the surface of the ground and then turned distad or upward a decimeter or more before making an exit. Several larvae were

found in their channels mining their way out above the ground during the early forenoon of May 21. Again late in the afternoon some were found with their heads protruding from exit holes. Many larvae were collected into fifty per cent. alcohol. They were from eleven to thirteen millimeters in length.

A large bundle of sprouts and seedling stems of *Prunus avium* and *P. domestica* 0.7–4 cm. in diameter was cut; and the ends of the sticks were stuck into vessels of soil. The soil in one vessel was made very wet and that in the other was about in condition for good tilth.

Several *P. domestica* and *P. avium* seedlings 1–4 cm. in diameter were found which had the cambium near the ground almost entirely destroyed by the numerous short-trip zigzag mines crossing and recrossing each other in that region.

On May 25 only about a sxith of the larvae were still in the host and by May 30 none were found under the bark; the mines were found occluded or closed. After a careful examination of the surface soil under clumps of *Prunus* sprouts and seedlings quite a number of small yellowish, somewhat fusiform and barrel-shaped pupae were found. Some were collected into alcohol. They were about 2.7 mm. in length and about 0.9 mm. in diameter (PLATE 10, FIG. 1). On examination of the surface soil in the vessels into which the *Prunus* stems had been inserted large numbers of the same pupae were found, indicating that they must be the pupae of the cambium mining larvae. In the vessel where the soil had been kept excessively wet a number of dead larvae were present, showing that excessive moisture prevents pupation in some cases. The stems were carefully removed and examined but no larvae could be found in them, while mines and exit holes were numerous. One of the vessels containing the pupae in its soil was placed in a cheese-cloth cage, and cheese-cloth was tied over the tops of the other vessels.

Pupae collected under *Prunus* shrubs on June 4 sank at once when dropped into alcohol while those collected on May 25 usually floated several minutes before sinking. The puparia seemed more tightly filled by the pupae on June 4 than before. On holding the vial containing pupae up to the light fully formed fly-like imagines could be distinctly seen. Twenty-five of the pupae collected under

Prunus shrubs were placed in soil in a breeding cage. On June 6 some more pupae were collected under both *Prunus* and *Crataegus* shrubs and placed in soil in the cheese-cloth cage. On June 14 they looked greyish black, apparently owing to the dark color of the insect inside.

On the morning of June 17 ten very small black flies were found flying about in the air space of a vessel over which cheese-cloth had been tied, and some were found walking about on the soil. Three were found dead and were placed in alcohol. They were about 2.5 mm. long. See FIGS. 2 and 3. Five flies (three females and two males) were placed into a glass tube containing the distal end of a *P. avium* shoot and the others into a quart fruit-jar which was inverted over a short green branch of *P. avium*.

Later in the day a search was made for the flies in the *Prunus* and *Crataegus* thickets but none could be found. However, quite a number of empty puparia were obtained from the soil beneath the shrubs. On June 21 all but one of the flies in the glass tube had died and it was placed in the fruit jar. The shoot in the tube was found to have groups of from one to four whitish eggs inserted under the periderm at the edge of a number of lenticels. Twenty eggs had been deposited under the periderm around the lenticels of this small twig by the three females caged with it.

A few flies emerged from the soil in the breeding cages each day until June 21 and were placed in vessels containing *Prunus* shoots; after that date all of the puparia were found empty. By June 25 most of the flies in the fruit-jar had died. The shoots were carefully examined and were also found to have eggs inserted under the periderm at many of the lenticels. Numerous larvae had already emerged from the eggs and were mining in the cortex while some had even mined as far as three centimeters in the cambium. This shows that oviposition and hatching of the eggs took less than a week. The eggs measured about 0.2×0.07 mm.

Seedlings and sprouts of *P. avium* and *P. domestica* in a thicket were found to have eggs inserted about numerous lenticels on June 21. In many cases the larvae had emerged from the eggs and were mining in through the cortex and cambium. But in no instance could more than one egg be found at a lenticel. It appears therefore that the deposition of eggs in the caged *Prunus* shoots

was so frequently found to be in groups because of the large number of flies present as compared to the few lenticels in which oviposition could occur. Both in nature and in the cages the deposition of the eggs seemed to have been accomplished by the insertion of the ovipositor through the lenticel; as may be inferred from FIG. 4. This is a photograph of a shoot from one of the breeding jars after removing the periderm from a portion of it.

The larvae always emerged from the end of the egg turned away from the lenticel and fed first in the cortex just under the periderm, mining parallel with the surface of the bark from half a millimeter to three millimeters; then straight in toward the cambium, where they continued their mines up or down depending upon how the eggs had been inserted. Most of the eggs were inserted so that their long axes coincided with that of the plant. In cases where the eggs had been inserted at an angle with the long axis the larvae always turned in the direction of the lesser angle with the stem, irrespective of whether it was up or down the plant. The direction of the first mine therefore depends upon oviposition and perhaps the direction of the long axes of the cambium cells encountered by the larva and is not uniformly up or down as suggested by Kienitz, Greene, and others. For example, in the case of those shown in the above cited figure there is a group of two eggs from which the larvae would have mined first toward the distal end of the shoot, while from the group of three they would have mined toward the base of the plant first. Some larvae were found which had mined as far as four decimeters in the cambium in one direction; others had mined only about a decimeter and then reversed the course and mined in the opposite direction, after having veered to one side from the old course. The emerging larvae were about 2–2.5 mm. long and not more than 0.2 mm. in diameter. They were comparatively numerous: four empty egg cases were found at lenticels of a piece of a *P. avium* twig which was only about six millimeters in diameter and three decimeters long. The larvae were collected into alcohol.

The egg stage of these cambium miners is very short, apparently less than three days; while the larval stage lasts at least eleven months. The pupal stage lasts perhaps about three weeks, and the flies apparently oviposit within two days after emerging from the puparia.

On July 16 the new generation of cambium miners was found to be rather few in number as compared with the large number of eggs and young that were present in late June. Possibly the excessive and very long drought in connection with the unprecedented hot weather in western New York during the latter part of June and the first part of July (1911) reduced cambial activity to such an extent as to make mining difficult and resulted in the death of many larvae.

COMPARISON OF THE PRUNUS AND OTHER DIPTEROUS CAMBIUM MINERS

When the life history of this *Prunus* miner is compared with that recorded by Nielsen for *Agromyza carbonaria* and with that given by Greene for *A. pruinosa*, as well as with the fragmentary history published by Kientz (discussed in my earlier paper), it becomes evident that though these miners are similar in many ways they must be different species. *A. carbonaria* and *A. pruinosa* pass the winter in the pupal stage in the ground while the *Prunus* miner goes through winter in the cambium of the host as a larva. The fact that the *Prunus* miner was never found in willows growing among infested *Prunus*, as recorded in my earlier paper, also goes to show that the insect so commonly present in *Salix* and *Betula* is not identical with the *Prunus* miner. In addition to the above noted and irreconcilable differences in the life histories and hosts of the miners there is a marked difference in the size of all the stages of the *Agromyza pruinosa* discussed by Greene and others and those of the one described in this paper. The most striking difference is to be found in the length of the larvae just before pupation: The measurements given by Greene and others for the fully grown larvae studied by them are 20–30 mm. while those of the fly under consideration here are only 11–13 mm. in length. The larvae of *A. carbonaria* also measure about 20 mm. in length; and besides, Nielsen calls especial attention to the reduction in the number of plate-girdles preceding pupation which does not hold of the miner of *Prunus*. The *Prunus* miner retains all the girdles at least to within a day of pupation. The adults of *A. carbonaria* and *A. pruinosa* as given by Nielsen and by Greene are also larger than these. The venation of the wings figured by

Nielsen for *A. carbonaria* and of *A. pruinosa* by Greene also differs from that of the wings of this species, as may be seen by comparing a, b, and c of FIG. 5. However, Brown's observations, published in the paper cited above, show beyond a doubt that the miner of the river birch also passes the winter as a larva in the cambium of the host, since on April 25 it was found feeding and measured 15 mm. in length. The *Prunus* miner appears to differ so materially from the other species in both its life history and morphology that I consider it best, at least provisionally, to name it **Agromyza Pruni** sp. nov.

THE OCCLUSION OF THE MINES AND THE NATURE OF THE WOUND-TISSUES PRODUCED

The configuration of the mines was described in some detail in my former paper but their occlusion and the reformation of the cambium over the streak-wound were only incidentally mentioned.

In sections showing mines near the cambium or within the unlignified zone one is able to see that a distinct new cambium develops over the channels by the division of the first to the third cell of the phloem outside the mine. FIGS. 6 and 7 give some idea of later but closely related stages. This new cambium is often found formed over the wounds before the regular cambial line is advanced that far, so that the cambium arches over the channels. FIG. 8 illustrates this point. The first noticeable change taking place in the cells surrounding a mine after the larva has passed is the rapid growth of the uninjured cells on the radial and bark sides of the channel. The phloem-ray cells usually enlarge more rapidly than the others and give rise to bladder-like proliferations which, after attaining considerable size, are cut off by septa from the cells giving rise to them (FIG. 9). The cells arising in this manner continue the growth in the same direction if space permits, and, if other proliferations do not crowd on the sides, their diameter becomes so great that septa soon form also parallel with the original direction of growth. See FIGS. 10 and 11. After the proliferating outgrowths from the rays encounter the opposite side of the mine the further enlargements are diverted in the directions of least resistance that are most nearly in line with their former direction of growth. In many instances of this type the cell back of the

terminal one undergoes lateral enlargement and proliferates into the available space, cutting off the outgrowth by a septum. An early stage of this process may be noted near the left of FIG. 9.

During the development of the substitute cambium over the streak-wound the growth of the phloem cells usually reduces the radial diameter of the mines from forty to sixty per cent. In the case shown in FIG. 12 this compression growth was so pronounced as to wholly prevent the usual form of occlusion. The phloem-ray cells appear to retard this general growth that brings about such a marked reduction of the channels, as may be seen from FIGS. 6 and 7 which show the status of things in an early stage. The broader rays have a more decided influence than the mono- or biseriate rays. As a matter of fact, the same characteristics may be noted in all the figures to a varying degree. The bark side of an occluded mine nearly always has a marked undulatory boundary line owing to the more active growth of the interray portions of the bounding phloem that occurs during the development of the substitute cambium over the wound. In some instances where channels were made through a region in which the rays are all monoseriate and at a time when radial growth is especially pronounced, they may be entirely closed by general growth from the bark and radial sides, thus leaving only the irregular lines of compressed waste and some irregularities in the arrangement of the cells to show the location of the medullary spot. That is, the growth of the interray portions dominates the healing process and thereby prevents proliferation from the rays, as shown in FIG. 12. The boundary line along the pith side of the occluded mine is usually fairly straight. This is especially noticeable in FIGS. 13 and 14. The tangential diameter of the occluded mine is from one to four or more times the diameter of the miner. This greater or tangential diameter of the original channel is also considerably reduced during the early stages of the healing process, as may be seen by the incurving of the bounding ray-cells, as well as by their bulging enlargements encroaching on the mined side. FIGS. 11, 15, and 16 show results under consideration. However, it is very unusual for the uninjured ray-cells on the sides to give rise to proliferations into the channels: the neighboring cells of the interrupted rays seem to respond more quickly, apparently owing to the

fact that longitudinal resistance or compression has been eliminated while in case of the others only the lateral pressure has been reduced. Neither diameter of a mine is increased by subsequent growth as suggested by Greene but all available evidence indicates that a considerable decrease occurs in both diameters during the occlusion of a cambium mine.

It appears that ordinarily the radial diameter of a channel is but little greater than that of the miner while the tangential diameter may be several times that of the larva. The principal reasons for this seem to be that the portion of the cambium most desired by the larvae for food is a sheath only a few cells in thickness and that they feed with the lateral sides toward the bark and wood. In that position the hooks with which the cell-walls are ripped open also lend themselves more readily to foraging a wide path in the plane of the cambium mantle than at right angles to it. The tangential widths of mines made by larvae of a certain size seem therefore to depend upon the relation of the movement and feeding impulses dominating them. When the cambial cells about a miner are in just the right stage to be most agreeable for food and the larva is hungry, and besides has no special desire to travel, it makes a very wide path; while if the cells encountered afford less suitable food or the insect is dominated by an impulse for movement rather than for feeding, the mine is made only large enough to permit its passage. That is, the mines have a greater tangential diameter at times chiefly because the larvae feed more voraciously and browse more widely at some times than at others. Compare the two medullary spots of FIG. 14: the lower resulted from a mine made in early summer, and the upper from one made in midsummer. Both appeared to have been made by the same larva, the lower one while the insect was younger and smaller.

The irregular mass of cells making up the tissue that occludes or fills up a channel left by a miner has the general appearance of pith. Like pith these occlusion masses come to serve as places for storing elaborated food. The chief difference between the appearance of sections from the two places lies in the greater variation in the size and shape of the medullary spot cells, as shown especially in FIG. 9. Sections of material collected in summer show that in general the cells occluding mines do not lignify

as soon as those of the normal tissue at an equal distance from the cambium; they also show that the cells making up medullary spots are not all of the same age nor produced under equal and like pressures: some of them lignify before others, some are angular while others are nearly circular in cross section. FIGS. 9, 13, and 14 show practically all of the types usually found.

The study of the development of such substitute tissues has much of indirect interest to plant pathology, and the wounds, when numerous, may sometimes not only prove very harmful to the host but incidentally afford entrance to parasitic organisms. In the case of *Prunus* no such connection with a fungous disease was established; a very striking example, however, was found in connection with the study of the *Ribes* cambium miner discussed in my earlier paper. In that case many of the shoots, vacated by a miner that it might pupate in the ground, were invaded by a fungus through the exit hole and killed back.

Description of plates 10 and 11

PLATE 10

FIG. 1. Pupae and puparium of the *Prunus* cambium miner (*Agromyza Pruni*). Upper right collected May 30; upper left collected June 4; lower right collected June 11; lower left collected after emergence of fly.

FIG. 2. Adult fly of *Prunus* miner (*Agromyza Pruni*) from breeding cage, 1911.

FIG. 3. Female with ovipositor extruded. From breeding cage, 1911.

FIG. 4. Twig of *Prunus avium* from breeding cage. The periderm removed to show location of two groups of eggs; the upper group had been inserted downward from lenticel above, and the lower one upward from lenticel below.

FIG. 5. Wings of various species of *Agromyza*. *a*, Wing of *A. carbonaria*, from Nielsen; *b*, Wing of *A. pruinosa*, from Greene; *c*, Photographed wing of *A. Pruni*.

FIG. 6. Medullary spot in *Prunus domestica*, near cambium; showing an early stage in the development of a new cambium from inner phloem cells.

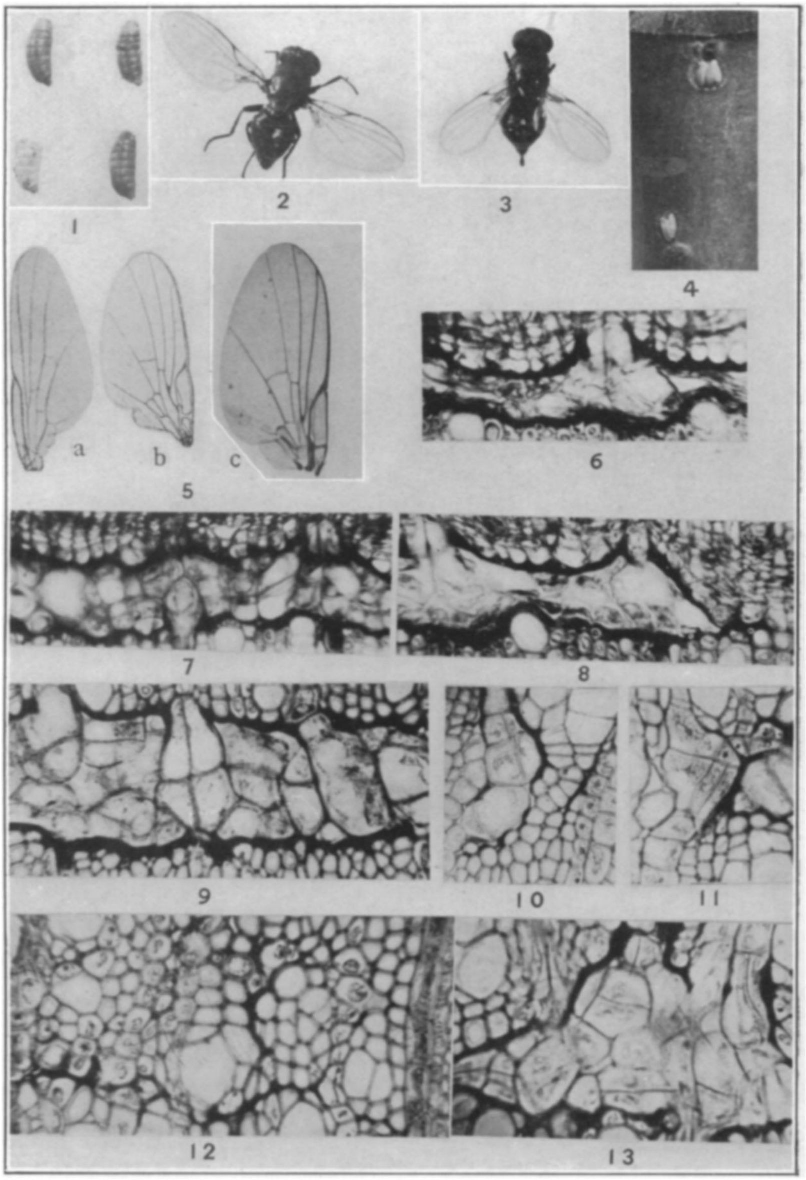
FIG. 7. Medullary spot in *Prunus domestica* near cambium, after the production of a few xylem cells outside the spot.

FIG. 8. Medullary spot with new cambium just developed outside the wound; being in advance of the other cambium as shown at right of figure.

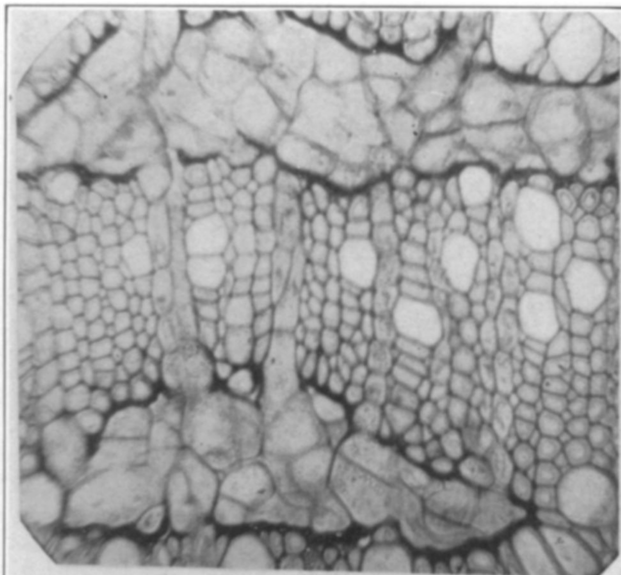
FIG. 9. Medullary spot in which the cells are not all mature, showing a marked variation in the size and shape of the cells.

FIG. 10. Medullary spot, made by a young larva and filled by proliferations from the rays and by the incurving of the radial rows on the sides as well as by the general growth on the bark side. (Upper.)

FIG. 11. Medullary spot, made by a young larva, filled by proliferation from ray on bark side and by bulging of ray from left side. The cells of the bulging ray are shown also to have undergone longitudinal division.



GROSSENBACHER: MEDULLARY SPOTS



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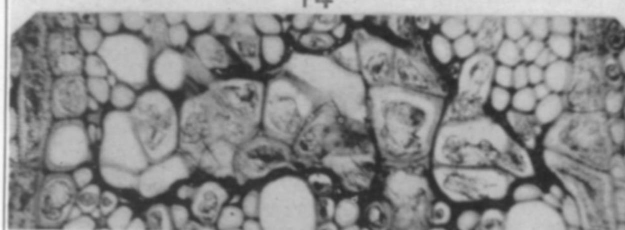

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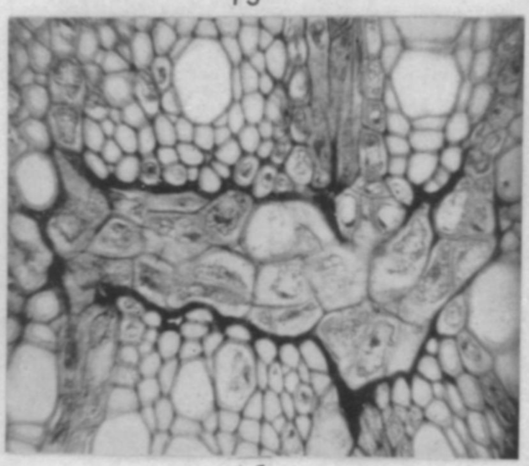

16

GROSSENBACHER: MEDULLARY SPOTS

FIG. 12. Medullary spot shown only by the presence of irregular lines of compressed waste. This channel was occluded at this point by general growth and not by proliferations from rays as is usually the case.

FIG. 13. Medullary spot, showing that bark rays not only proliferate freely into mines but that they *thereby* prevent or retard the general compression growth, that is especially pronounced at the left where large rays are absent.

PLATE 11

FIG. 14. Two medullary spots, apparently made by the same miner, also showing well marked differences between boundary lines on the bark and pith sides of the occluded channel.

FIG. 15. Medullary spot showing incurving of bounding rays at right and left, as well as a bulging of the ray at right.

FIG. 16. Medullary spot showing incurving of rays and of other radial rows.